REMARKS

Claims 1, 4, 7, 8, 9, 11, 12 and 27 have been amended. Support for the amendments can be found in the Specification ad claims as originally filed, for example in paragraphs [0068] ad [0079], and in claims 2, 3, 5, and 6. No new matter has been introduced by these amendments. Claims 2, 3, 5, 6 have been canceled as redundant in view of amendments to Claim 1, and Claim 10 has been canceled without prejudice. The following addresses the substance of the Office Action.

Definiteness

The Examiner has rejected Claim 10 under 35 USC §112, second paragraph as indefinite. Specifically, it was not clear which data would be collected. In view of amendments to Claim 1, Claim 10 has been canceled as redundant. Therefore, this rejection is now moot.

Enablement

The Examiner has rejected Claims 1 and 15 under 35 USC §112, first paragraph as allegedly non-enabled. Specifically, the claims are currently restricted to measuring an aspect of cell activation by studying three cellular proteins in particular, namely CDK8, PAK6 and MKK3. The Specification, however, provides working examples performed with Akt, Erk and p38. The Examiner further stated that CDK8 is unique among CDKs in that appears that its activation is not dependent on phosphorylation as is observed with other CDK family members. The Examiner cited Hoeppner et al. (2005 *J. Mol. Biol.* 350:833-842), who determined that CDK8 is lacking the threonine in its activation loop that is present in other CDKs, and that is phosphorylated to activate such CDKs. Rather, CDK8 is activated by interaction of the residue that replaces the otherwise phosphorylated threonine with its binding partner Cyclin C. Therefore, the Examiner stated that no evidence was provided in either the instant specification for determination of activation of CDK8 by phosphorylation, while there is evidence in the literature that phosphorylation of CDK8 is not an effective means for determining cellular activation state. The Examiner further noted that the specification does not provide working examples of phospho-specific antibodies for the elected species CDK8 and PAK6, and therefore is not sufficiently detailed to enable a skilled artisan to perform a cell activation assay using phosphorylation of these signalling molecules as a readout. Applicant respectfully disagrees.

The presently claimed invention is based on a comparison of the total versus phosphorylated amount of proteins, with CDK8 (cyclin-dependent kinase 8), MKK3 and PAK6 taken as example.

CDK8 is involved in the cellular transcriptional machinery, together with CDK7 and CDK9. CDKs are activated by different mechanisms. For example, CDK2, the most studied member, requires phosphorylation of Thr160 for maximal activation. Such a threonine residue is absent from CDK5, nevertheless Sharma et al. (*PNAS USA* 96:11156-11160, 1999) showed that the CDK5 catalytic activity is regulated through phosphorylation on a serine residue. CDK9 also lacks a threonine at a position equivalent to the Thr160 in CDK2, but its phosphorylation on threonine and serine residues present on a C-terminal tail, absent in CDK2, was proved crucial for its catalytic activity (Garber et al. 2000 *Mol. Cell. Biol.* 20:6958-6969). CDK8 is the biggest CDK known so far, and its structural conformation is likely different from that of CDK2. It lacks the Thr160 residue, but harbors a C-terminal tail like CDK9, which contains several Ser and Thr residues. CDK8 also shares some functional similarities with CDK9 (Gold & Rice, 1998 *Nucleic Acids Res.* 26(16):3784-3788). The activation of CDK8 may therefore well be dependent on phosphorylation events on residues different from Thr160.

Hoeppner et al. have determined the 3D structure of Cyclin C; and generated an interaction model with its CDK8 partner based on homology modelling where they used the smaller CDK2 as a model. This theoretical study can not predict or exclude the CDK8 regulation by phosphorylation, which can only be demonstrated experimentally. Accordingly, the authors only suggest alternative activation mechanisms. But determining the phosphorylation status of CDK8 by a method of the invention absolutely makes sense in view of the integrated results collected on other CDKs, as it very likely participates in the protein activation mechanism.

MKK3 belongs to the MAPK cascade. It is a substrate for several kinases such as Ask1 and Tak1 and phosphorylates p38 for which working examples are provided. As such, it is regulated by phosphorylation like p38, and is well adapted to the present method.

PAK6 is also regulated by phosphorylation (Jaffer & Chernoff, 2002 *Int. J. Biochem. Cell. Biol.* 34(7):713-717) ant its phosphorylation state may well inform about the activation status of target cells.

All phosphorylatable proteins may be analyzed by the method according to the present invention. Detailed information about respective phosphorylation targets and locations of phosphorylation sites may be *inter alia* obtained from the National Center for Biotechnology Information (NCBI) database. The skilled person is very well capable of selecting and preparing suitable protein fragments, non-phosphorylated and in different phosphorylation statuses, from said fragments either by protein synthesis and subsequent phosphorylation or by isolating the fragments from cells in different stages (such as different life stages or disease stages) in which proteins of the cells may be in an activated or non-activated state. In a further step antibodies against said fragments may be raised, which antibodies may be prepared according to standard procedures. Since several suitable antibodies are already commercially available and may be purchased from various companies.

Accordingly, the skilled person is enabled by the present specification how to perform an array experiment according to the present invention for determining the phosphorylation status of the 3 phosphorylatable kinases CDK8, MKK3 and PAK6.

Novelty

The Examiner has rejected Claims 1, 2, 11, 12, 14, and 22-25 under 35 USC §102(b) as being allegedly anticipated by Paweletz et al. (2001).

> To be anticipatory under 35 U.S.C. § 102, a reference must teach each and every element of the claimed invention. *See Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1379 (Fed. Cir. 1986). "Invalidity for anticipation requires that all of the elements and limitations of the claim are found within a single prior art reference. ...There must be no difference between the claimed invention and the reference disclosure, as viewed by a person of ordinary skill in the field of the invention." *See Scripps Clinic & Research Foundation v. Genentech, Inc.*, 927 F.2d 1565 (Fed. Cir. 1991).

Paweletz et al. do not teach contacting a biological sample with two arrays, wherein a first array is used to immobilize only phosphorylated cellular proteins, while the second array is used to immobilize the proteins regardless of their phosphorylation state, and then quantifying the level of phosphorylation of said immobilized proteins by measuring the signal ratio between the phosphorylated versus the total proteins present in the sample. Furthermore, Paweletz does not teach that the target molecules (proteins) are bound to the capture probes via sites or epitopes not bearing a phosphate moiety.

Therefore, Paweletz et al. do not teach all the limitations of the currently amended Claim 1, and thus do not anticipate currently amended claims 1, 2, 11, 12, 14, and 22-25.

The Examiner has rejected Claims 1, 2, 13, 22, 26 and 27 under 35 USC §102(b) as being allegedly anticipated by Lee et al. (2001).

Lee et al. do not teach contacting a biological sample with two arrays, wherein a first array is used to immobilize only phosphorylated cellular proteins, while the second array is used to immobilize the proteins regardless of their phosphorylation state, and then quantifying the level of phosphorylation of said immobilized proteins by measuring the signal ratio between the phosphorylated versus the total proteins present in the sample. Furthermore, Lee does not teach that the target molecules (proteins) are bound to the capture probes via sites or epitopes not bearing a phosphate moiety. Therefore, Lee et al. do not teach all the limitations of the currently amended Claim 1, and thus do not anticipate currently amended claims 1, 2, 11, 12, 14, and 22-25.

For all of the above reasons, Applicants respectfully request withdrawal of all rejections under 35 U.S.C. § 102, and allowance of the pending application.

Non-obviousness

The Examiner has rejected Claims 1-9, 11, 12, 14, 15, 22-27 and 29 under 35 USC §103(a) as being allegedly unpatentable over Paweletz et al. (2001) in view of Huang (2001). Specifically, the Examiner stated that it would have been obvious at the time the invention was made to a person of ordinary skill in the art to use protein-specific antibodies immobilized onto a support as capture molecules in the method of determining phosphorylation state of candidate proteins from differentially treated cell lysates taught by Paweletz et al., because Huang teaches hat it is possible to study differences in individual proteins by spotting their antibodies onto a support and capturing specific proteins for studying posttranslational modifications (e.g. protein phosphorylation).

> To establish a *prima facie* case of obviousness a three-prong test must be met. First, there must be some suggestion or motivation, either in the references or in the knowledge generally available among those of ordinary skill in the art, to modify the reference. Second, there must be a reasonable expectation of success found in the prior art. Third, the prior art must reference must teach or suggest all the claim limitations. *In re Vaeck*, 947 F.2d 488 (Fed. Cir. 1991).

The present invention is based on the realization that the mere quantification of one phosphorylated protein or the detection of several phosphorylated proteins by itself is not sufficient to determine, whether the cell is in an activated state or not and is surely not sufficient to estimate the level of the activation, since some proteins are phosphorylated due to a general basal physiological activation, resulting in a given basal phosphorylation of some proteins in any case. According to the presently claimed invention, the quantitative phosphorylation level of any target protein or any plurality of target proteins present in cell is assessed by comparing the amount of phosphorylated specific cellular proteins versus the total amount of said proteins (see, for example, paragraphs [0060]-[0061]).

The combination of Paweletz et al. with Huang does not render obvious the claimed subject matter because such combination does not provide suggestion or motivation to modify either of the references, and cited references do not teach or suggest all the claim limitations. Paweletz et al. describes making microarrays of immobilized protein lysates from histopathological cell population. In this reverse phase protein blot, the complex experimental sample itself is printed and probed with detection molecules, and then compared to another sample (for example, control). The cellular lysates are immobilized on nitrocellulose slides with a glass backing. In contrast to antibody array, there is no need for a capture probe immobilized on the solid support of Paweletz et al. The method described allows a rapid screening of many samples but is highly subject to the specificity of the detecting molecules. For this purpose, the specificity of each detection antibody is checked by Western blot (see page 1988, left column, fourth paragraph). Also, given the small surface area of microarray spots, there is a limited number of available sites for printed proteins. When printing a complex protein mix in this context, abundant proteins may interfere with capture of other, less abundant proteins, limiting the dynamic range and preventing the assay of rare proteins on the array surface. Nevertheless, given these limitations, reverse phase protein blots finds their greatest application in screening experiments across many experimental samples for a handful of well-defined proteins, which will then require a more detailed and quantitative analysis using other methods. Accordingly, Paweletz et al. do not give any hint of how to compare the amount of phosphorylated specific cellular proteins versus the total amount of said proteins <u>in the same cell extract</u>. The use of capture molecules is a prerequisite to provide quantitative measurements of two forms of a same

protein, i.e. the total and phosphorylated forms as described in the present invention. Capture molecules indeed immobilize target proteins in a homogeneous fashion, by epitopes or sites which leave the phosphate moieties available for their subsequent detection.

Huang also does not teach the detection and quantification of phosphorylated proteins and the total amount of the proteins in the same cell extract. Furthermore, in contrast to the presently claimed method in which the capture polypeptide sequences are bound to a non-membrane solid support, Huang teaches the use of antibody or antigens spotted onto membranes in an array format. The binding of the proteins on membrane means that there is a diffusion of the protein within the membrane network. As a consequence, there is no control of which part of the protein would be available for reaction with the cellular protein and the speed of reaction would vary from one spot to the other. In contrast to the method of Huang, the utilization of non-membrane solid support in the present method ensures that the portions of the capture molecules which are specific to the cellular proteins are available for the reaction. Moreover, the absence of diffusion of the cellular proteins into the support ensures a comparable speed of reaction with the immobilized capture molecule whatever their position in the array. The reproducibility of such binding reaction is crucial for the quantitative determination of the total amount of specific cellular proteins and the amount of phosphorylated proteins.

Therefore, the combination of Paweletz et al. with Huang et al. does not provide motivation to modify either of the references to achieve the claimed method, and this combination does not teach all the limitations of the currently amended Claim 1. Therefore, Paweletz in combination with Huang does not render Claims 1-9, 11, 12, 14, 15, 22-27 obvious.

The Examiner has rejected Claims 1-4 under 35 USC §103(a) as being allegedly unpatentable over Paweletz et al. (2001) in view of Huang (2001), and further in view of Gustafson et al. (US 5,413,939) or Chang et al. (US 2002/0192654A1). Specifically, the Examiner stated that because Gustafson et al. describe a method of binding antigens or antibodies to a compact disc, or because Chang et al. describes that a biochip is broadly defined in the art as a product for immobilizing DNA, protein or cell structure on glass, silicon or plastic plate for biochemical analysis, it would have been obvious to one of ordinary skill in the art at the time the invention was made to use a plastic, glass, or silicon support in a method of measuring phosphoproteins for the purpose of determining cell activation.

Non-obviousness of the currently amended Claim 1 over the combination of Paweletz and Huang is asserted above. The additional references of Gustafson or Chang do not cure the deficiencies of the two primary references. Therefore, currently amended Claims 1 and 4 are non-obvious over the cited combination of references.

The Examiner has rejected Claims 22 and 28 under 35 USC §103(a) as being allegedly unpatentable over Paweletz et al. (2001) in view of Matsui et al. (*EMBO J.* 1996). Specifically, the Examiner stated that it would have been obvious at the time the invention was made to a person of ordinary skill in the art to use the method of Matsui et al. of isolating lysate-kinase activity first and then applying a test compound in the method of testing phosphorylation equilibrium by binding phosphoproteins to a solid support. However, Matsui et al. does not cure the main deficiencies of Paweletz et al. as asserted above. Therefore, the combination of these references does not render Claims 22 and 28 obvious.

For all of the above reasons, Applicants respectfully request withdrawal of all rejections under 35 U.S.C. § 103, and allowance of the pending application.

CONCLUSION

Applicants have endeavored to address all of the Examiner's concerns as expressed in the outstanding Office Action. Accordingly, amendments to the claims, the reasons therefor, and arguments in support of the patentability of the pending claim set are presented above. In light of the above amendments and remarks, reconsideration and withdrawal of the outstanding rejections is specifically requested. If the Examiner finds any remaining impediment to the prompt allowance of these claims that could be clarified with a telephone conference, the Examiner is respectfully requested to initiate the same with the undersigned.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

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